

interaction between the target protein, and the ligand is not modified, and wherein a response transduction function is not modified, the fluorescent protein being chosen from the fluorescent proteins obtained or derived from autofluorescent proteins of cnidarians, the molar extinction coefficient of which is greater than about  $14,000 \text{ M}^{-1}\text{cm}^{-1}$  and the quantic fluorescence yield is greater than about 0.38, this protein being selected from the group consisting of green fluorescent protein (GFP), variants of GFP that conserve the fluorescence property, and fragments of GFP that conserve the fluorescence property, placing said cells or said cell fragments in contact with a ligand for said target protein, said ligand labeled with a label comprising a molecule capable of absorbing the light emitted by the fluorescent protein, or a fluorescent substance, the fluorescent protein being the fluorescence energy donor and the label being the fluorescence energy acceptor, or the fluorescent protein being the fluorescence energy acceptor and the label being a fluorescent substance which is a fluorescence energy donor, and irradiating said cells or said cell fragments at a wavelength which makes it possible either to excite the fluorescent protein or to excite the fluorescent substance, wherein the steps of placing in contact and irradiating are carried out either simultaneously or one after the other, or said cells or said cell fragments are placed in contact with a ligand for said protein, said ligand labeled with a label, the cells or the ligand having been irradiated before being placed in contact,

- wherein a reduction in the amplitude of the donor's emission and/or emission signal characteristic of the acceptor's emission is detected and optionally measuring the fluorescence energy transfer when quantifying the non-covalent interactions.

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--37. (new) Process according to Claim 36, further comprising:

- before, after or simultaneously with the step for placing in contact fluorescent protein fused with the target protein and said ligand for said protein, said ligand being labeled with a label, or

- before, after or simultaneously with the step of placing in contact said cells or cell fragments and said ligand for said protein, labeled with a label,

this additional step consisting:

- placing the said fluorescent protein fused with the target protein in contact with a non-labeled ligand and simultaneously with said labeled ligand,

- or placing said cells or said cell fragments in simultaneous contact with said non-labeled ligand and said labeled ligand,

- either a decrease in the amplitude of the donor's emission and/or an emission signal characteristic of the acceptor's emission is detected, respectively, in the case of using the labeled ligand and in the case of simultaneously using the labeled ligand and the non-labeled ligand,

- and either the reduction in the amplitude of the donor's emission obtained and/or the emission signal characteristic of the acceptor's emission obtained are compared.

--38. (new) Process according to Claim 37, wherein the fluorescent protein is EGFP and wherein:

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- either the EGFP is a fluorescence energy donor and the label is a fluorescence energy acceptor and is chosen from substances whose excitation spectrum overlaps the emission spectrum of EGFP, and in particular, when the label is a fluorescent substance, it is chosen from: 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (Bodipy), eosin, erythrosin, tetramethylrhodamine, sulphorhodamine 101 sold by Molecular Probe under the name Texas Red, and derivatives thereof which, on the one hand, allow grafting, and, on the other hand, have an excitation spectrum which overlaps the emission spectrum of EGFP,

and, when the label is not a fluorescent substance, it is chosen from the Acid Violet group [Acid Violet 5, CAS 10130-48-0; Acid Violet 7, CAS 4321-69-1; Acid Violet 17, CAS 4129-84-4], the Acid Red group [Acid Red 1, CAS 3734-67-6; Acid Red 8, CAS 4787-93-3; Acid Red 37, CAS 6360-07-2; Acid Red 40, CAS 12167-45-2; Acid Red 106, CAS 6844-74-2; Acid Red 114, CAS 6459-94-5], alizarins, aluminon, azocarmine B [CAS 25360-72-9], basic fuschin [Basic Red 9, CAS 569-61-9], Bordeaux R [Acid Red 17, CAS 5858-33-3] and Carmine [CAS 1390-65-4],

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- or the EGFP is a fluorescence energy acceptor and the fluorescent substance is a fluorescence energy donor and is chosen from substances whose emission spectrum overlaps the excitation spectrum of EGFP selected from the group consisting of coumarins, fluorescamine, 6-(N-methylanilino)naphthalene, (mansyl) and derivatives thereof.

--39. (new) Process according to Claim 36, wherein the protein whose protein-ligand interaction is selected from the group consisting of:

- membrane-bound proteins coupled to the G protein,
- growth factor receptors which are structurally linked to the insulin receptor,
- ion channel-receptors, and
- intracellular nuclear receptors which are structurally linked to the steroid receptor.

--40. (new) Process according to Claim 36, wherein the fluorescent protein is EGFP and the labeled substance is Bodipy and in which either the reduction in the emissions amplitude of EGFP or an emission signal of Bodipy resulting from an energy transfer is detected, the irradiation wavelength corresponding to the excitation wavelength of EGFP.

--41 (new) Process according to Claim 36, wherein the fluorescent protein is EGFP and the labeled substance is a coumarin, and wherein either the diminution of amplitude of coumarin or an emission signal EGFP resulting from an energy transfer is detected, said irradiation wavelength corresponding to the excitation wavelength of coumarin.

--42 (new) Process according to Claim 36, wherein the fluorescent protein is fused on the N-terminal side and the target protein is fused on the C-terminal side.

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--43 (new) Process according to Claim 36, wherein the fluorescent protein is fused on the C-terminal side and the target protein is fused on the N-terminal side.

--44. (new) Process according to Claim 36, wherein the fluorescent protein is inserted into a receptor target protein wherein said receptor is coupled to the G protein, this insertion taking place in the first or third intracellular loop of the receptor, with the proviso that the insertion does not destroy either the properties of the receptor or the fluorescence of the fluorescent protein.

--45. (new) Process according to Claim 36, wherein the cells are mammalian cells, in particular HEK 293 cells which are adherent or in suspension selected from the group consisting CHO cells, COS cells, lymphocytic lines, fibroblasts, yeast cells *pichia pastoris*, *saccharomyces cerevisia*, *saccharomyces kluyveri*, *Hansenula polymorpha*, insect cells infected with a virus and fungi.

--46. (new) Process according to Claim 36, wherein a signal can be detected, in a conventional fluorimetry device or in a rapid-mixing device equipped with a system for detecting fluorescence, after mixing the donor and the acceptor, and can be abolished by the addition of a non-fluorescent substance of similar pharmacological specificity, and wherein a signal/noise ratio is greater than about 2.

--47. (new) Process for detecting and/or quantifying non-covalent interactions between a target protein consisting of a receptor coupled to G proteins and a G protein, in order to identify the molecules which are biologically active with respect to the receptor, and which are capable of forming a reversible non-covalent interaction with said receptor, wherein:

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- cells or fragments of cells which express a DNA sequence comprising the gene coding for a fluorescent protein fused with the gene for the receptor coupled to the G proteins are prepared, the fusion between the gene coding for the fluorescent protein and the gene for said receptor being that the properties of the receptor are not modified by the presence of the fluorescent protein, namely:

- \* the interaction between the receptor and the G protein is not modified,
- \* the interaction between the receptor and the biologically active molecule is not modified,
- \* a response transduction function is not modified, the fluorescent protein being chosen from the fluorescent proteins obtained or derived from autofluorescent proteins of cnidarians, the molecular extinction coefficient of which is greater than about  $14,000 \text{ M}^{-1}\text{cm}^{-1}$  and the quantic fluorescence yield of which is greater than about 0.38, this protein being chosen in particular from:
  - green fluorescent protein (GFP), or
  - variants derived from GFP by addition, deletion or

substitution of one or more amino acids, with the proviso that these variants conserve the fluorescence property,

- or fragments of GFP, or fragments of the above-mentioned variants, with the proviso that these fragments conserve the fluorescence property,

the G protein being labeled with a label consisting:

- either of a molecule which is capable of absorbing the light emitted by the fluorescent protein,

- or of a fluorescent substance,

- the fluorescent protein and the above-mentioned label being such that they transfer energy from one to the other, it being possible for the fluorescent protein to be an energy donor or it being possible for the above-mentioned label to be an energy donor,

detecting the interaction between the receptor labeled with the fluorescent protein and the G protein labeled with said label by fluorescence energy transfer and optionally measuring the fluorescence energy transfer when quantifying the non-covalent interactions.

--48. (new) Process for identifying and/or quantifying interactions between a receptor and a non-fluorescent molecule which is biologically active with respect to the said receptor, which are capable of forming a reversible non-covalent interaction with said receptor, by implementing the process defined according to claim 47, in which a biologically active non-fluorescent molecule is added to cells, or cell fragments, which express the DNA coding for the receptor labeled with the fluorescent protein

and for the G protein labeled with the label, characterized in that:

- an agonist and biologically active non-fluorescent molecule triggers a signal transduction detected by variation in the energy transfer between the receptor labeled with the fluorescent protein and the G protein labeled with the label;

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- an antagonistic biologically active non-fluorescent molecule inhibits the signal transduction brought about by an agonist and detected by variation in the transfer of fluorescence energy between the receptor labeled with the fluorescent protein and the G protein labeled with the label.

--49. (new) Kit or equipment for detecting and/or quantifying non-covalent interactions between a target protein labeled with a fluorescent protein and one of its ligands labeled with a label consisting:

- a molecule which is capable of absorbing the light emitted by the fluorescent protein,

- or a fluorescent substance,

this fluorescent protein being chosen from the fluorescent proteins obtained or derived from autofluorescent proteins of cnidarians, the molecular extinction coefficient of which is greater than about  $14,000 \text{ M}^{-1}\text{cm}^{-1}$  and the quantic fluorescence yield of which is greater than about 0.38, this protein being further chosen from green fluorescent protein (GFP), variants of GFP that conserve the fluorescence property, and fragments of GFP and said variants, that conserve the fluorescence property and its ligand labeled with a fluorescent substance, the



said kit comprising:

- the target protein fused with a fluorescent protein or a stable cell line which is capable of expressing the protein fused with a fluorescent protein or a plasmid containing the nucleic acid sequence coding for the said targets protein fused with a fluorescent protein as defined above,

- the ligand labeled with the above-mentioned label,

- buffers and media required for the energy transfer between said protein and said ligand.

--50. (new) Kit or equipment for detecting and quantifying non-covalent interactions between a target protein labeled with a first fluorescent protein and one of its ligands labeled with a fluorescent substance corresponding to a second fluorescent protein, said first fluorescent protein being chosen from the fluorescent protein EYFP or EGFP and the ligand being labeled with said second fluorescent protein ECFP, or said first fluorescent protein being ECFP and the ligand being labeled with said second fluorescent protein EYFP or EGFP, the said kit comprising:

- a plasmid containing a nucleic acid sequence coding for the target protein fused with a fluorescent protein, and a plasmid containing a nucleic acid sequence coding for the ligand fused with a second fluorescent protein, or a ligand fused with a second fluorescent protein, obtained via a recombinant route and purified, or

- a stable cell line which is capable of expressing the target protein fused with first fluorescent protein, and a stable

cell line which is capable of expressing the ligand fused with a second fluorescent protein or a ligand fused with a second fluorescent protein, obtained via a recombinant route and purified, and

- buffers and media required for an energy transfer between said protein and the said ligand.

51. (new) Kit or equipment for detecting and quantifying non-covalent interactions between a target protein consisting of a receptor coupled to the G protein labeled with a first fluorescent protein and the G protein labeled with a fluorescent substance corresponding to a second fluorescent protein, the first fluorescent protein being chosen from the fluorescent protein EYFP or EGFP and the G protein being labeled with the second fluorescent protein ECFP or the first fluorescent protein being ECFP and the G protein being labeled with the second fluorescent protein EYFP or EGFP, the said kit comprising:

- a plasmid containing a nucleic acid sequence coding for the receptor fused with a first fluorescent protein, and a plasmid containing a nucleic acid sequence coding for the G protein fused with a second fluorescent protein, or the G protein fused with a second fluorescent protein, obtained via a recombinant route and purified; or

- a stable cell line which is capable of expressing the receptor fused with a first fluorescent protein, and a stable cell line which is capable of expressing the G protein fused with a second fluorescent protein, or the G protein fused with a second

fluorescent protein second , obtained via a recombinant route and purified; and

- buffers and media required for the energy transfer between the above-mentioned receptor and the above-mentioned G protein.

--52. (new) Process for detecting and/or quantifying non-covalent interactions between a target protein and one of its ligand, characterized in that:

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*Q2*

- a fluorescent protein fused with a target protein, the protein-ligand interaction of which it is desired to determine, is prepared, the fusion between the fluorescent protein and said target protein being such that the interaction between the target protein, in particular the receptor, and the ligand is not modified, and the response transduction function is not modified, the fluorescent protein being chosen from the fluorescent proteins obtained or derived from autofluorescent proteins of cnidarians, the molecular extinction coefficient of which is greater than about  $14,000 \text{ M}^{-1}\text{cm}^{-1}$  and the quantic fluorescence yield of which is greater than about 0.38, this protein being further chosen from green fluorescent protein (GFP), variants of GFP that conserve the fluorescence property, and fragments of GFP and of said variants that conserve the fluorescence property,

- said fluorescent protein fused with the target protein is placed in contact with a ligand of the above-mentioned protein, this ligand being labeled with a label comprising a molecule which is capable of absorbing the light emitted by the fluorescent protein, or a fluorescent substance, the fluorescent protein being

a fluorescence energy donor and the label being a fluorescence energy acceptor, or the fluorescent protein being a fluorescence energy acceptor and the label being a fluorescent substance which is a fluorescence energy donor, and

- irradiation is carried out at a wavelength which makes it possible either to excite the fluorescent protein or to excite the fluorescent substance,

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- it being possible for the above-mentioned steps of placing in contact and irradiation to be carried out either simultaneously or one after the other, or

- said fluorescent protein fused with the target protein is placed in contact with a ligand for the above-mentioned protein, this ligand being labeled with a label comprising a molecule which is capable of absorbing the light emitted by the fluorescent protein, or a fluorescent substance, the fluorescent protein fused with the target protein or the ligand having been irradiated before being placed in contact,

- wherein a reduction in the amplitude of the donor's emission and or an emission signal characteristic of the acceptor's emission is detected and optionally measuring the fluorescence energy transfer when quantifying the non-covalent interactions.--

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R E M A R K S

At the outset, Applicants would like to thank Examiner Sisson for his time and consideration of the present application at the interview of March 26, 2002 with the undersigned Agent.